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Hydration State, Metabolism, and Hatching of Mono Lake *Artemia* Cysts

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Abstract. *Artemia monica*, the only macrozooplankton in Mono Lake, California, is unique among brine shrimp in that it produces encysted diapause embryos that sink to the lake bottom where they overwinter. Currently, the lake's salinity is about twice as high as it was 40 years ago and, at equilibrium, it is projected to fluctuate between 169–248 g/l. Here we describe the effects of salinity on the termination of diapause, hatching, carbohydrate metabolism, and hydration of the cysts. As expected, hatching is much more sensitive to salinity than is termination of diapause. Carbohydrate metabolism, which involves the conversion of trehalose to glycerol and is required for hatching, responds to increasing salinity as reported in other *Artemia* species: increasing amounts of glycerol must be synthesized as salinity is raised. The unfreezable water in these embryos is 0.29 g H_2O/gram dry weight (gdw) cysts, similar to values reported for other biological systems. This result and previous studies suggest that water probably becomes limiting at hydration levels of about 0.60 g H_2O/gdw cysts. In Mono Lake water, the cysts reach this critical hydration at a salinity between 140–160 g/l, equivalent to approximately 3780–4330 mOsm/kg. We conclude that *Artemia monica* will cease to exist within this salinity range and doubt that it can hatch beyond this limit, which is imposed by the requirement of metabolic processes for minimal amounts of cellular water.

Introduction

The relationships between external salinity, metabolic activity, and the physical state of cellular water have been studied extensively in the encysted diapause embryo of *A. franciscana* (Clegg, 1964, 1978, 1986; Glasheen and Hand, 1989). In their natural environment, the cysts are frequently subjected to extreme fluctuations in salinity and complete desiccation. Rupture of the cyst wall during hatching is thought to be an osmotic process, brought about by the synthesis of glycerol and the resulting increase in turgor pressure (Clegg, 1964, 1976a). Although the cysts can hatch in a wide range of salinities by increasing the amount of glycerol produced as salinity increases, they reach a point where hatching is completely inhibited due to inadequate cellular water (Clegg, 1964).

The critical hydration levels that limit metabolism in *A. franciscana* have been investigated by Clegg and colleagues (1974, 1976a, b, c; 1977; Clegg and Cavagnaro, 1976; Clegg and Lovallo, 1977). In an elegant series of studies, they report that the shutdown of metabolism due to water loss occurs in a step-wise fashion, with distinct metabolic transitions corresponding to changes in the physical state of water remaining in the cysts. The metabolic characteristics and hydration levels of these three metabolic domains are shown in Table I. Cysts with water contents lower than about 0.60 H_2O g/gdw (gram dry weight) exhibit a dramatic decrease in their metabolic capabilities. Further studies suggest that a significant bulk aqueous phase is not present until the cysts contain more than 0.6 g H_2O/gdw. Clegg has hypothesized that, as hydration levels fall below 0.60 g H_2O/gdw, metabolic pathways are disconnected, resulting in a restricted metabolism that does not permit hatching of the cyst (for reviews of the model see Clegg, 1978, 1986). Another metabolic transition occurs at hydration levels of 0.3 g H_2O/gdw and lower. This water is considered to be the "bound water," and at water contents of 0.3 g/gdw and lower, the only metabolic activity evident is a slow decline in ATP.

An atypical species of brine shrimp, *A. monica*, inhabits Mono Lake, California—a large, deep, termial lake on the eastern side of the Sierra Nevada. Unlike *A. francis-*

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Table I

Hydration-dependence of cellular metabolism in Artemia *cysts*

Cyst hydration (g H_2O/gdw cysts)	Metabolic events initiated
0 to 0.1	None observed
0.1	Decrease in ATP concentration
0.1 to 0.3 ± 0.05	No additional events observed
0.3 ± 0.05	Metabolism involving several amino acids, Krebs-cycle and related intermediated, short chain aliphatic acids, pyrimidine nucleotides, slight decrease in glycogen concentration
0.3 to 0.6 ± 0.07	No additional events observed
0.6 ± 0.07	Cellular respiration, carbohydrate synthesis, mobilization of trehalose, net increase in ATP, major changes in the free amino acid pool, hydrolysis of yolk protein, RNA and protein synthesis, resumption of embryonic development
0.6 to 1.4	No additional events observed

The two critical levels of hydration where large changes in metabolic capacity occur are shown in bold typeface. After Clegg (1978).

cana cysts, Mono Lake cysts are not subjected to desiccation, and rarely experience drastic changes in salinity. The thin cyst shell permits the cysts to sink to the lake bottom where they are activated (diapause is terminated) by the cold temperatures. They remain on the lake bottom for 6–7 months, until late February to early March, when they begin hatching (Lenz, 1980, 1983).

The future viability of this species is of concern, because water exports from the Mono Lake basin have caused a decline in the lake level. The lake's salinity has more than doubled over the past 40 years and, at equilibrium, it is predicted to fluctuate between 169 and 248 g/l (Vorster, 1985). The shrimp are the only macrozooplankton in the lake, and serve as a food source for the large population of California gulls that have a major rookery on the lake. Thus, *A. monica* occupies a key position in the Mono Lake ecosystem (Mason 1967; Winkler, 1977; Lenz, 1980).

This study examines the effects of salinity of the metabolism and hydration of the cysts in *Artemia monica*. The results indicate that in terms of their response to elevated salinity, *A. monica* is very similar to the well-studied brine shrimp, *A. franciscana*, despite pronounced differences in the habitats of these two species.

Materials and Methods

Collection of cysts

Gravid females collected from Mono Lake were held in the laboratory for 1–2 weeks while they released their cysts. Conditions approximated those of Mono Lake in the summer: temperature was 18°C, and Mono Lake water (MLW) containing 50 g solids/l (1300 mOsm/kg) was used; no food was provided. Before being used, the cysts were stored anaerobically in this medium at 14°C. The newly released diapause cysts will not hatch, even when placed in conditions normally favorable for hatching; they require a cold treatment (≤5°C) of about 90 days (Dana, 1981; Drinkwater and Crowe, 1987). Storage at 14°C, as described above, had previously maintained the diapause state and yielded viable cysts (Drinkwater and Crowe, 1987).

Osmolality of Mono Lake water

The physiologically relevant measurement of the dissolved solids in this water is osmolality, as we will show in the Results section. However, previous workers have represented their results in terms of grams of dissolved solids per liter of water (g/l). To facilitate a comparison of the present results with those of previous studies, we will report data here in both forms. Furthermore, previous workers have referred to the measurement g/l as "salinity" even though this is not the precise oceanographic meaning of this word. We will continue this usage.

It is not possible to reconstitute Mono Lake water from the salts collected by complete evaporation because some insoluble salts are formed during precipitation. Consequently, we produced the desired salinities by partially evaporating water collected from the lake and determining the salt content of subsamples gravimetrically. Adjustments were made to the non-desiccated stock by dilution with distilled water to yield MLWs of varying salinity. The osmolality of these solutions was determined by measurements of freezing point depression. Samples of the solutions were frozen in an ethylene glycol bath chilled to about −20°C. The temperature was monitored, and the equilibrium freezing point was recorded during the release of the latent heat of fusion as the samples froze. These measurements were repeated four times on each sample.

Metabolic studies

Preliminary analyses indicated that the carbohydrate profile of *A. monica* cysts was essentially identical to those of other *Artemia* cysts: prior to development the embryos contained high levels of trehalose and glycogen, and low levels of glycerol. Therefore, we expected that trehalose would be mobilized for glycerol production during pre-emergence development (PED).

In the first experiment, we determined when trehalose degradation is initiated. Diapause cysts were incubated aerobically and anaerobically at two temperatures, 14°C and 4°C. We knew from our previous work that the cysts

in the 14°C incubations would not break diapause and, consequently, would not hatch. However, the 4°C treatment would permit the cysts to break diapause, and they would begin hatching if adequate oxygen were present (Drinkwater and Crowe, 1987). The inclusion of the anaerobic treatments allowed us to break diapause but inhibit hatching, as aerobic metabolism is obligatory for hatching to occur (Clegg and Conte, 1980). Because the cysts could break diapause and hatch in the aerobic 4°C incubation, we set up parallel groups such that the percent hatch under these conditions could be monitored (Drinkwater and Crowe, 1987). This first experiment enabled us to compare the carbohydrate metabolism of cysts remaining in diapause (14°C) to that of cysts which had terminated diapause (4°C) and resumed development.

The effects of salinity on carbohydrate metabolism were studied as follows. Diapause cysts were incubated aerobically at 4°C in MLW of four salinities: 50, 80, 100, and 125 g/l (1300, 2100, 2690, and 3370 mOsm/kg). Two petri dishes of cysts were set up at each salinity and maintained in hygrostats over water of the same salinity. The media were monitored with a refractometer to assure constant salinity. Under these conditions, cysts can break diapause and begin hatching, thus percent hatch was monitored as described above. In addition, to separate the effects of salinity on termination of diapause from those on hatching, subsamples were periodically taken and placed under favorable hatching conditions: 14°C, in MLW of 50 g/l (1300 mOsm/kg).

Carbohydrate assays

Samples of cysts were removed and decapsulated by exposure to 2% hypochlorite (diluted household bleach), at 4°C, until examination under a dissecting microscope showed that the cyst shell had been removed (Sorgeloos *et al.*, 1977). This usually required 5 to 10 min. Each sample was divided into three subsamples and weighed after desiccation over $CaSO_4$. Trehalose, glycerol, and glucose were extracted by grinding cysts in a tissue homogenizer in 60% ethanol. Soluble sugars were separated by high pressure liquid chromatography (HPLC) on a HPX-87H anion exchange column (Bio-Rad; Schwarzenbach, 1982) and were quantified with a Knauer differential refractometer. The pellet was analyzed for glycogen according to the anthrone method (Umbreit *et al.*, 1972).

Calorimetry: determination of unfreezable water

A Perkin-Elmer DSC2-C Differential Scanning Calorimeter, supplemented with a Perkin-Elmer 3600 data station and TADS thermal analysis software, was used to determine the amount of unfreezable water in hydrated cysts of *A. monica* and *A. franciscana*. Decapsulated cysts were hydrated to varying degrees, either by submersion in distilled water, or by exposure to water vapor in individual hygrostats (Clegg, 1974). Cysts hydrated in the liquid phase required thorough blotting to remove all water on their surfaces. We sometimes observed two endothermic spikes due to water on the surface of the cysts; these samples were discarded.

The majority of the data presented in this paper are from cysts hydrated from the vapor phase. Samples (8 mg) were placed in pre-weighed aluminum calorimetry pans and sealed. The pans were weighed and the amount of freezable water was then measured by freezing the cysts, allowing them to reach thermal equilibrium at −63°C, and running calorimetry scans from −63°C to 27°C.

Frozen water in the cysts was quantified by comparing the enthalpy of the melting endotherm for water in the frozen cysts (calculated by the Perkin-Elmer TADS software), with enthalpy of known standards treated in the same way. After calorimetry, the pans were punctured to permit desiccation of the cysts by lyophilization. The samples were reweighed after equilibrating over $CaSO_4$. The water content of the samples was determined as the difference between the wet and dry weights.

Hydration of cysts in Mono Lake water

The water content of cysts as a function of the salinities of MLW was determined according to the method of Clegg (1974). Cysts were placed in individual chambers constructed of 35 ml covered vials and hydrated from the vapor phase over MLW of 50, 80, 100, 125, 140, 160, and 200 g/l. Six days were needed for cysts in the vapor phase to reach equilibrium at 2°C (Clegg, 1974). After being weighed, the cysts were lyophilized and then brought to equilibrium over $CaSO_4$ for 10 h in individual desiccators; the dry weight was then determined.

Results and Discussion

Salinity of Mono Lake water: a clarification

The salinities of MLW and its dilutions have, in the past, been compared with those of solutions of entirely different ionic compositions such as seawater or NaCl solutions (Dana, 1981; Dana and Lenz, 1986). Mono Lake water has an unusual salt composition, with high levels of carbonates and sulfates (Cole and Brown, 1967). Because the cysts respond to the chemical potential of water, we clearly cannot make direct comparisons of solutions containing different ionic species on a g/l basis. Such comparisons have led to considerable confusion in the literature. Our careful measurements of the osmolality of diluted Mono Lake water and NaCl solutions illustrate this point (Fig. 1). The osmolality of MLW is lower than a solution of NaCl containing the same amount of salts by weight. Thus, the water content of cysts in Mono Lake

water is higher than those in the NaCl solution containing the same amount of salts on a g/l basis. This seemingly simple point is exceedingly important, in that interpretation of data based on dissolved solutes can lead to incorrect conclusions, as illustrated by the following discussion.

Previous workers have shown that *A. monica* can hatch in MLW of 133 g/l (Dana and Lenz, 1986). Limits for many *Artemia* populations are between 70–99 g NaCl/l, and because the highest salinity for hatching (*A. franciscana*, Utah population) is reported as >99 g NaCl/l (d'Agostino, 1965, as cited by Collins, 1977), Dana and Lenz concluded that *A. monica* is unusual in its ability to hatch at increased salinities. However, Figure 1 shows that 133 g/l MLW is equivalent to a NaCl solution of about 105 g/l (3500 mOsm)—very similar to the highest reported salinity permitting hatching in *A. franciscana*. We conclude that *A. monica* is not unusual with regard to its hatchability as a function of osmolality of the bathing solution.

Salinity effects on the hatching mechanism in A. monica

As shown in Figure 2, breakdown of trehalose into glycerol only occurs under aerobic conditions in cysts that are able to break diapause and hatch. Therefore, the same osmotic mechanism proposed for hatching in *A. franciscana* is likely present in *A. monica*.

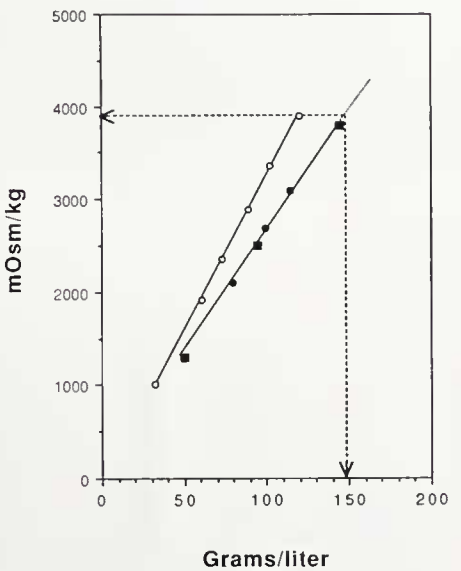

Figure 1. Osmolality of NaCl solutions and Mono Lake water of equal salt content on a g/l basis. Dotted arrows indicate that a 2.0 *M* (117 g/l) NaCl solution is the osmotic equivalent of about 150 g/l Mono Lake water. NaCl data are from Weast (1983). Data for Mono Lake water are from two sources using different methods: (squares) our data, obtained by freezing point depression; (circles) Herbst and Dana (1980), determined by vapor pressure osmometry.

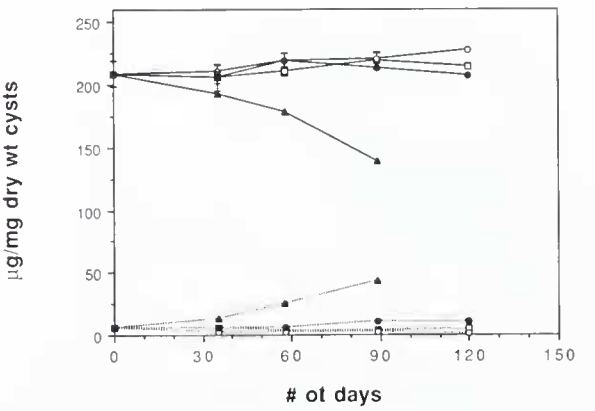

Figure 2. Trehalose (solid lines) and glycerol (broken lines) contents of cysts incubated under the following conditions: 4°C, aerobic (triangles); 4°C, anaerobic (closed circles); 14°C, aerobic (open circles); 14°C, anaerobic (squares). The 4°C, aerobic incubation is the only treatment which permitted both activation and hatching; the cysts were not sampled after 90 days because the majority of them had hatched. Note the significant decline in trehalose as glycerol increases. The other three incubations exhibited no hatching; cysts in the 4°C, anaerobic incubation could break diapause, but could not hatch in the absence of oxygen. Points are $\bar{x} \pm SD$, $n = 3$.

Having shown that the synthesis of glycerol proceeds with hatching, we incubated cysts in several salinities of MLW to determine the effect of salinity on carbohydrate metabolism. Simultaneously, we monitored termination of diapause and hatching. Increasing salinities resulted in faster synthesis of glycerol (Fig. 3a), while trehalose breakdown is slower at higher salinities (Fig. 3b). Figure 3c shows that, at the lowest salinity (50 g/l), glycogen is synthesized in addition to glycerol, suggesting that at this salinity some of the trehalose is being converted to glycogen. However, glycogen shows net degradation in the higher salinities, indicating that an osmotic regulatory mechanism may control the amount of glycerol and glycogen synthesized by the embryo in response to changes in salinity. The decline of glycogen may, in part, also explain the lower hatch seen in Figure 4 at higher salinities. If the embryo must synthesize more glycerol to hatch as salinity increases, fewer carbohydrate reserves will be available for other necessary developmental processes.

Salinity effects on termination of diapause

Figure 5 indicates that there is only a slight inhibition of diapause termination in the highest salinity used in these experiments. The percent hatch is essentially the same in three of the salinities, with only a 20% lower hatch in the cysts from the 125 g/l (3370 mOsm/kg) treatment. When data from the metabolic studies are combined with the hatching data from this study and others (Dana and Lenz, 1986), we can conclude that the decrease

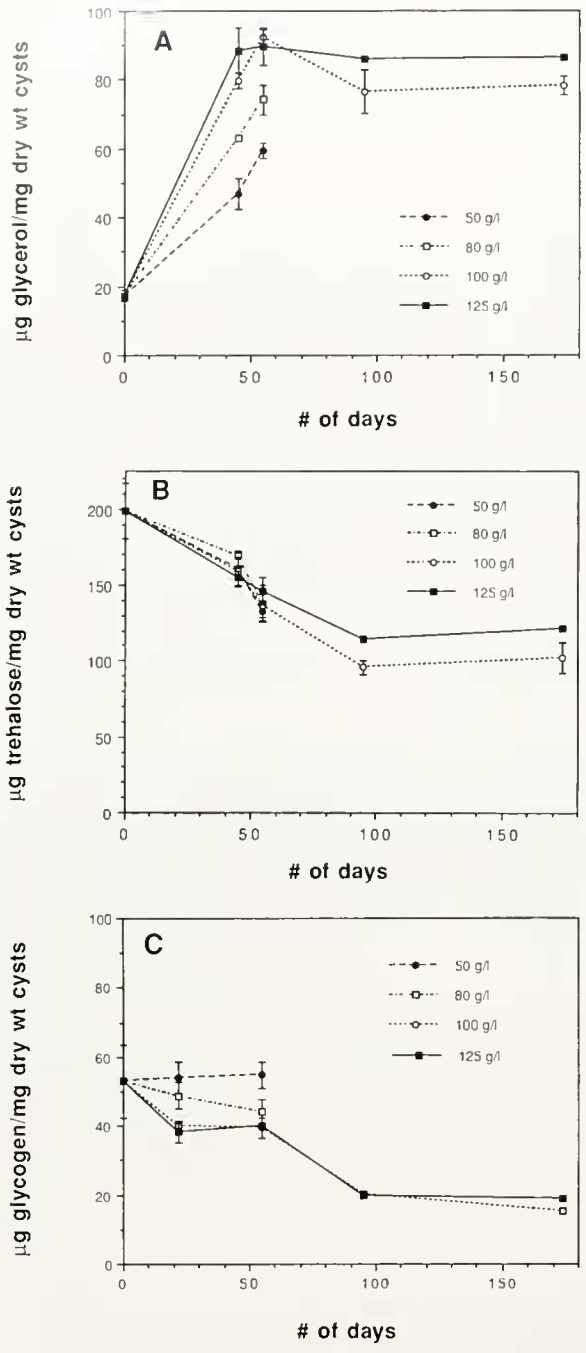

Figure 3. Changes in carbohydrate levels of cysts incubated aerobically in four salinities of Mono Lake water at 4°C. (A) Glycerol synthesis is faster at higher salinities. (B) Trehalose breakdown is faster in the lower salinities. (C) Glycogen levels; only the lowest salinity, 50 g/l (1300 mOsm/kg), shows a net increase in glycogen. Points are x ± SD, n = 3. Refer to Figure 4 to determine percent hatch during the experiment.

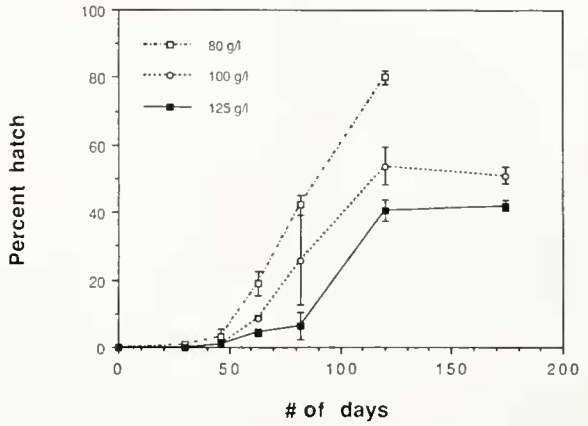

Figure 4. Percent hatch of *Artemia monica* during the experiment described in Figure 3. Hatching in the 50 g/l (1300 mOsm/kg) treatment was essentially the same as the 80 g/l (2100 mOsm/kg) treatment and is not shown. Each point represents x ± SD, n = 3.

in hatching observed at the higher salinities used in these experiments primarily results from interference with hatching rather than release from diapause. Thus, termination of diapause is less susceptible to increasing salinities, because salinities that only impair cyst activation completely inhibit hatching (Dana and Lenz, 1986; Drinkwater and Crowe, 1987).

Unfreezable water and hydration of Mono Lake cysts

Previous experiments indicate that the hatching limit of *A. monica* is somewhere between 133–159 g/l MLW (Dana and Lenz, 1986). Based on our metabolic data and studies of the hydration dependence of metabolism, we assume that, at this limiting salinity, conventional metabolism can no longer occur, and degradation of trehalose therefore stops. However, the potential for adaptations permitting the cysts to hatch at higher salinities is a possibility to which several researchers working at Mono Lake have alluded (Dana, pers. comm.). We have attempted to

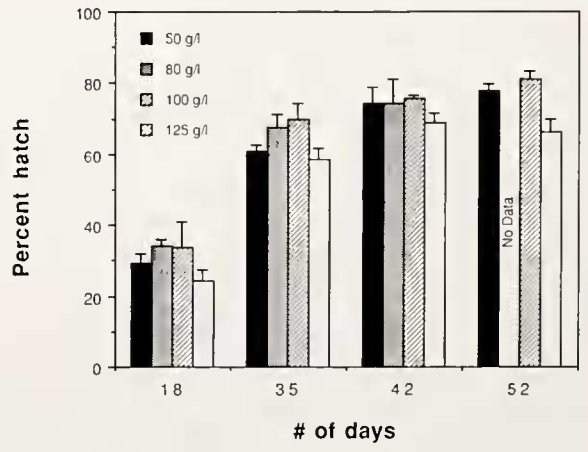

Figure 5. Percent hatch of cysts removed from the treatment salinity and hatched in 50 g/l (1300 mOsm/kg) at 14°C to determine the number of activated cysts. x ± SD of three determinations are graphed.

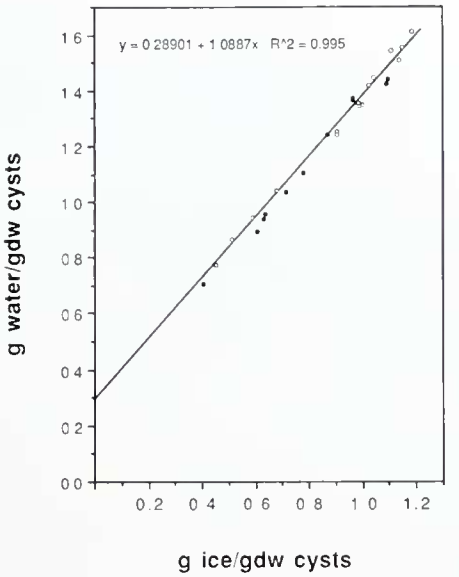

Figure 6. Unfreezable water in *Artemia monica* (open circles) and *A. franciscana* (closed circles). The line represents a linear regression on the *A. monica* data; the *A. franciscana* data are included for comparison. The y-intercept (unfreezable water) is 0.29 g H_2O/gdw cysts.

determine the physical limitation of conventional metabolism in these organisms by studying their hydration properties and unfreezable water content.

The amount of unfreezable water in *A. monica* cysts corresponds closely with our data for *A. franciscana* (Fig. 6). The y-intercept gives the estimated amount of unfreezable water, in this case, 0.29 g H_2O/gdw for *A. monica* cysts. A few *A. franciscana* samples were run for comparison. Linear regression of these points estimates unfreezable water to be 0.28 g/gdw cysts, very close to our value for *A. monica*. These values for unfreezable water coincide closely with the critical hydration at which the transition to the ametabolic state occurs, about 0.3 g/gdw (Table I; Clegg, 1978, review). Below this water content, the cysts are considered to be ametabolic. Thus, the unfreezable water content represents a physiologically significant hydration feature of the cells.

Comparing the quantity of bound water contained in these two species (0.28–0.29 g/gdw) with figures reported for a wide range of biological systems, we find close agreement; amounts range from 0.3 to 0.5 g/gdw (Williams, 1970; Cooke and Kuntz, 1974, Garlid, 1978; O'Dell and Crowe, 1979; Crowe *et al.*, 1983). Our results do not agree with previous findings for a now extinct population of *A. franciscana* previously located in Brazil; freezable water in that study was about 0.6 g/gdw (Crowe *et al.*, 1981). However, because the phase transition curve for the *A. franciscana* cysts was reported to be curvilinear rather than linear, the cysts probably had water in their outer porous shells, causing the internal water content to appear higher than it actually was. In the previous experiments with *Artemia* (Crowe *et al.*, 1981), the cysts were hydrated by immersion in water, whereas in our present experiments, hydration was achieved by exposure to the vapor phase, eliminating this potential source of error.

Table II

Water content (g H_2O/grams dry weight) of decapsulated Artemia monica *cysts in Mono Lake water of salinities ranging from 50 to 200 g/l*

Salinity (g/l)	Cyst hydration g H_2O/gdw cysts
50	1.16 ± 0.06
80	1.04 ± 0.08
100	0.97 ± 0.15
125	0.76 ± 0.01
140	0.66 ± 0.02
160	0.55 ± 0.04
200	0.39 ± 0.02

Data reported as $\bar{x}$ ± S.D., n = 3.

Salinity and water content of Mono Lake cysts

Finally, the water contents of *A. monica* cysts in varying concentrations of Mono Lake water were determined to assess the point at which hatching would be limited by insufficient intracellular water. In Table II, the hydration levels of *A. monica* cysts equilibrated in MLW of 50–200 g/l are reported. To permit comparison with *A. franciscana*, previously published data have been corrected for the presence of the shell and have been graphed with *A. monica* in Figure 7; a close correspondence in water contents is demonstrated. *A. monica* cysts reach a critical hydration of 0.6–0.67 g/gdw in salinities between 140–

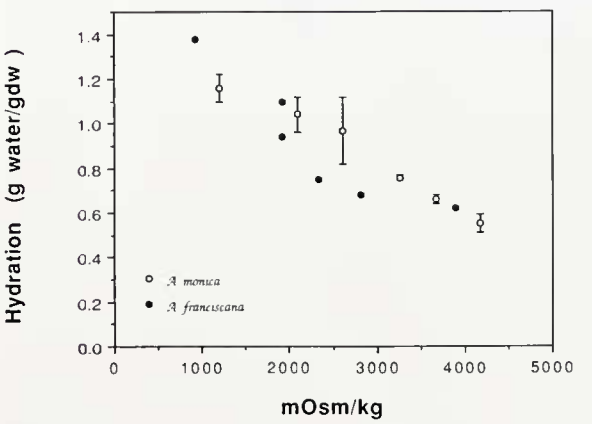

Figure 7. Hydration of *Artemia monica* cysts compared to *A. franciscana* cysts as a function of osmolality. Data from *A. franciscana* have been corrected for shell weight (from Clegg, 1974, 1976a).

160 g/l MLW, equivalent to NaCl solutions of approximately 1.9–2.1 *M* (3600–4050 mOsm/kg).

Clegg (1978) has not detected conventional metabolism in *A. franciscana* cysts hydrated in 2.0 molal NaCl. More recently, Glasheen and Hand (1989) have used microcalorimetry to demonstrate that heat dissipation, and thus metabolism, in *A. franciscana* from the Great Salt Lake is severely depressed by 2.0 *M* NaCl. We suggest that *A. monica* also experiences a critical hydration at this salinity, and submit that conventional metabolism will not occur in these cysts at a limiting salinity between 140–160 g/l MLW. We must stress, however, that at salinities somewhat lower that these, which actually impose a physical limit to conventional metabolism, hatching will be impaired; *i.e.*, a smaller proportion of cysts will hatch, and hatching will take longer (Jennings and Whitaker, 1941; Clegg, 1964; Dana and Lenz, 1986).

Conclusions

Several lines of evidence presented here suggest that cysts of *A. monica* possess limits to development that are similar to those found in *A. franciscana.* (1) Hatching is correlated with the synthesis of glycerol and, as in *A. franciscana,* synthesis of this compound is probably required for hatching. Glycerol synthesis increases when the cysts are incubated at higher salinities. (2) The amounts of unfreezable water in *A. monica* and *A. franciscana* are similar, suggesting that the hydration levels at which metabolic transitions occur are the same. (3) At salinities of about 150 g/l MLW (equivalent to 4060 mOsm/kg), the water content of *A. monica* cysts is less than 0.6 g/gdw, thus conventional metabolism and development will not be possible.

We conclude that, while *A. monica* can hatch in salinities in the upper range of those reported for *Artemia* cysts, they are not unique in this ability, and they have no unusual adaptive potential with respect to salinity thresholds. All available evidence suggests that these limits on metabolism are imposed by the biophysical interactions inherent in the hydration of cellular components and the effects of this water of hydration on the functioning of macromolecular assemblages (Clegg, 1986; Glasheen and Hand, 1989).

Thus, these organisms probably cannot adapt to Mono Lake salinities above about 150 g/l (4060 mOsm/kg) by extending their hatching limit beyond that level. Biological adaptation, powerful as it is, cannot overcome the basic principles of eukaryotic metabolism which require the presence of minimal amounts of cellular water. It follows then, that *A. monica* will become extinct when salinity rises to between 140–160 g/l—even before the lake reaches equilibrium. Certainly, *A. monica* will not exist in Mono Lake when it reaches its projected equilibrium, since salinity will then be 169–248 g/l, well above 4000 mOsm/kg (Fig. 1).

Finally, should the salinities in Mono Lake be allowed to reach these levels, we doubt that another brine shrimp species could be successfully introduced due to the characteristics of this lake that make it unique among *Artemia* habitats (Lenz, 1980; Dana, 1981; Bowen *et al.*, 1985; Drinkwater and Crowe, 1987). First, because of its ionic composition, Mono Lake water is toxic to many *Artemia* populations, including the most well-known North American species, *A. franciscana* (Bowen *et al.*, 1985). Second, in order for a species to persist in the lake, its life-cycle would need to be synchronized with the conditions in Mono Lake; *i.e.*, diapause induction and termination must occur at the appropriate times. In addition, the Mono Lake ecosystem probably cannot mimic other hypersaline lakes, such as the Great Salt Lake, in which floating cysts are deposited on the shore as the lake recedes and are then swept back into the lake by spring rains. The average annual precipitation in the Mono basin is 33 cm, compared to 52 cm for the Great Salt Lake. And in the spring, average precipitation for Mono Lake is only 4 cm (April, May, and June), while the Great Salt Lake receives 16 cm during these same months (NOAA, 1985, 1986). These observations illustrate some of the specific difficulties involved in attempting to introduce a replacement brine shrimp species into Mono Lake.

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